REMARKS

The foregoing amendments to the specification correct minor typographical errors. Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made.**" It is respectfully submitted that no new matter is added and that no issues relating to patentability are raised by this Amendment. This Amendment is submitted simultaneously with payment of the issue fee. Entry of this Amendment under 37 CFR §1.312 is thus respectfully requested.

Respectfully submitted,

Date March 8, 2003

By _____
Gregory T. Pletta
Attorney for Applicant
Registration No. 47,864

Please send all correspondence to:

Gregory T. Pletta
EraGen Biosciences, Inc.
918 Deming Way, Ste. 201
Madison, WI 53717

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph that immediately follows the Background of the Invention heading, at page 2, line 2, has been amended as follows:

Natural oligonucleotides bind to complementary oligonucleotides according to the well-known rules of base pairing first elaborated by Watson and Crick, where adenine (A) pairs with thymine (T) or uracil (U), and guanine (G) pairs with cytosine (C), with the complementary strands anti-parallel to one another. These pairing rules allow for the specific hybridization of an oligonucleotide with complementary oligonucleotides, making oligonucleotides valuable as probes in the laboratory, in diagnostic applications, as messages that can direct the synthesis of specific proteins, and in a wide range of other applications well known in the art. Further, the pairing is the basis by which enzymes are able to catalyze the synthesis of new oligonucleotides that are complementary to template nucleotides. In this synthesis, building blocks (normally the triphosphates of ribo or deoxyribo derivatives of A, T, U, C, or G) are directed by a template oligonucleotide to form a complementary oligonucleotide with the correct sequence. This process is the ~~bases~~ basis for replication of all forms of life, and also serves as the basis for all technologies for enzymatic synthesis and amplification of specific heterosequence nucleic acids by enzymes such as DNA and RNA polymerase, and in the polymerase chain reaction.

The paragraph beginning line 21, page 6, has been amended as follows:

Statements considering non-standard base pairs in a general way can, to our knowledge, be found only four times previously in the literature. Considering possible bases that might have been incorporated into nucleic acids in the first forms of life on the earth two to four billion years ago, Rich mentions the base pair between iso-C and iso-G (Rich, A. (1962), Horizons in Biochemistry, Kasha, M. and Pullman, B. editors, N.Y., Academic Press, 103-126) as a base pair that was conceivable, but rejected, by the earliest forms of life. However, Rich did not disclose nor make obvious the method disclosed here where oligonucleotide strands containing non-standard bases would recognize complementary oligonucleotides incorporating the base pair between iso-C and iso-G into oligonucleotides. Indeed, shortly before, Dekker suggested in a review article that the iso-C nucleoside is chemically unstable (Dekker, C. (1960) Ann. Rev. Biochem. 464). Saenger (Saenger, W. (1985) Nucleic Acid Chemistry, Springer-Verlag) also mentions this base pair, but concludes, based on the fact that iso-G has ~~a~~ alternate tautomeric forms (vide infra), that it has no utility as part of an oligonucleotide that is to be copied.

The paragraph beginning line 7, page 7, has been amended as follows:

Zubay (Zubay, G. (1988) The Roots of Modern Biochemistry, Kleinkauf, von Doehren, Jaenicke, Berlin, Walter de Gruyter & Co. 911-916) suggested that 2,4-diamino-5,6-~~dihydropyrymidine~~dihydropyrimidine-1-riboside, with a donor-acceptor-donor pattern, might be able to pair with xanthosine. In Zubay's suggested pyrimidine, however, the pyrimidine ring is not aromatic and therefore not planar. Although it has never been examined experimentally, we believe on these grounds that it would not participate well in "base stacking," the interaction (vide supra) that is important for the stability of a double helix. Further, Zubay's base incorporates the structural unit known as a "vinylogous enamine", a structural unit that is likely to be unstable in acidic solution. Thus, we doubt that it can be incorporated into an oligonucleotide by enzymatic transcription of a complementary oligonucleotide.

The paragraph beginning line 1, page 8, has been amended as follows:

Additional literature has discussed nucleosides that could perform as a component of a non-standard base pair, but evidently without the realization that non-standard base pair is possible. For example, iso-G is a component of the natural product crotonoside, which is a ribonucleoside analog. The chemistry of the iso-G riboside was studied in the 1970's by Shugar and his coworkers (Golar, T., Fikus, M., Kazimierczuk, Shugar, D. (1976) Eur. J. Biochem. 65, 183-192; Sepiol, J., Kazimierczuk, Z., Shugar, D. (1976) Naturforsch. 31c, 361). ~~Chu etal.~~ Chu et al. reported the synthesis of a pyrimidine donor-acceptor-donor non-standard nucleoside (Chu, C. K., Reichman, U., Watanabe, K. A., & Fox, J. J. (1977) J. Org. Chem. 42, 711-714), but again without reference to potential Watson-Crick base pairing. Further, we are unaware of the preparation of deoxynucleoside derivatives bearing these non-standard nucleobases. Oligodeoxyribonucleotides, because of the absence of 2'-hydroxyl groups, are more stable to hydrolytic cleavage than the corresponding oligoribonucleotides. Similar stability can be achieved with 2'-O-methoxyribonucleotides, as is well known in the art.

The paragraph beginning at line 22, page 9, has been amended as follows:

Finally, and most generally, non-standard bases incorporated into oligonucleotides might provide a molecular recognition system that has the "rule-based" behavior of DNA and RNA, but which does not bind to complementary DNA and RNA from natural systems. Such a molecular recognition system should have ~~used~~ use in building nanostructures, in diagnostics, and in forensic medicine.

The paragraph beginning at line 13, page 29, has been amended as follows:

2-Amino-3-(5'-tert.-butyldimethylsilyl-2',3-O-isopropylidene-β-D-ribofuranosyl)-5-methylpyrazin-1-oxide. 2-(5'-tert.-Butyldimethylsilyl-2',3'-O-isopropylidene-β-D-ribofuranosyl)-N-Cbz-D,L-glycinenitrile (100 mg, 0.21 mmol) was dissolved in dioxane (2 mL). 10% Pd-C (10 mg) was added and the suspension stirred at RT in an H_2-atmosphere over night. The Pd-C was removed by centrifugation. Anti-methylglyoxal-1-oxime (27 mg, 0.31 mmol) was added, the solution heated to reflux for 54 h and the solvent evaporated. Chromatography (silica gel, EtOAc/hexane 8:2) yielded 2-amino-3-(5'-tert.-butyldimethylsilyl-2',3'-O-isopropylidene-β-D-ribofuranosyl)-5-methylpyrazin-1-oxide (40 mg, 46% for two steps) as a brown oil. A larger scale reaction (23 mmol) ~~proceeded~~ proceeded analogously. MS m/z (rel intensity) 411 (M^+; 1), 208 (92), 150 (55), 134 (57), 133 (71), 117 (53), 75 (100), 73 (99); IR ($CHCl_3$): 3430, 3320, 2990, 2960, 2930, 2860, 1610, 1565, 1490, 1385, 1335, 1260, 1140, 1105, 1080, 985, 860, 840; ^{1}H-NMR ($CDCl_3$): 0.06 (s, 3H, Me-Si), 0.09 (s, 3H, Me-Si), 0.83 (s, 9H, tBu), 1.39 (s, 3H, Me_2C), 1.62 (s, 3H, Me_2 C), 2.37 (s, 3H, Me-C(5)), 3.82 (dd, J=2.3, 11.3, 1H, H-C(5a')), 3.91 (dd, J=2.6, 11.3, 1H, H-C(5b')), 4.24 (m, 1H, H--C(4')), 4.83 (dd, J=3.6, 6.5, 1H, H-C(3')), 5.02-5.09 (m, 2H, H-C(1') and H-C(2')), 6.28 (s, 2H, NH_2), 7.87 (s, 1H, H-C(6)); ^{13}C-NMR ($CDCl_3$): -5.53 (q, Me_2Si), 18.48 (s, tBu), 20.52 (q, Me-C(5)), 25.48 (q, Me_2C), 25.90 (q, tBu), 27.45 (q, Me_2C), 62.81 (t, C(5')), 80.99, 82.93, 85.24 and 87.40 (d, C(1'), C(2'), C(3') and C(4')), 114.88 (s, Me_2C), 129.13 (d, C(6)), 139.45, 141.26 and 143.59 (s, C(2), C(3) and C(5)).

The paragraph beginning at line 13, page 50, has been amended as follows:

8-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-4-[(dimethylamino)methyliden]-imidazo[1,2-a]-1,3,5-triazin-2(8H)-one. 8-(β-D-2'-Deoxyribofuranosyl)-4-[(dimethylamino)methyliden]-imidazo[1,2-a]-1,3,5-triazin-2(8H)-one (75 mg, 0.23 mmol) was dried over P_2O_5 under high vacuum for 2 days and then dissolved with warming in abs. DMF (3.5 mL). The soln. was cooled to ~~r.t.~~RT, and a soln. of dimethoxytrityl chloride (103 mg, 0.32 mmol) dissolved in abs. pyridine (0.9 mL) was added. After stirring for 9.5 h at ~~r.t~~RT., the reaction was quenched with a mixture of MeOH/pyridine 1:1 (0.2 mL), and the solvents removed under high vacuum. Chromatography through silica gel (10 g)(eluant: CH_2Cl_2 /MeOH 100:1 then 100:5 then 100:10, each containing 1% Et_3N) yielded 8-[2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-4-[(dimethylamino)methyliden]-imidazo[1,2-a]-1,3,5-triazin-2(8H)-one (127 mg, 66%) as a microcrystalline solid with a trace of Et_3N as impurity. This was used directly without further purification. FAB-MS (3-NOBA): 625 (M^++1); ^{1}H-NMR ($CDCl_3$): 2.43-2.48 (m, 1H, Ha-C(2')), 2.76-2.81 (m, 1H, Hb-C(2')), 3.18 (d, J=0.6, 3H, N-Me), 3.22 (s, 3H, N-Me), 3.28 (dd, J=3.7, 10.5, 1H, Ha-C(5')), 3.42 (dd, J=3.0, 10.5, 1H, Hb-C(5')), 3.78 (s, 6H, O-Me), 4.23-4.24 (m, 1H, H-C(4')), 4.70-4.73 (m, 1H, H-C(3')), 5.45 (s, br., 1H, OH), 6.48 (dd, J=6.2, 6.3, 1H, H-C(1')), 6.80-6.98 (m, 4H, ortho-arom. H), 7.04 (d, J=2.8, 1H, H-C(6) or H-C(7)), 7.10 (d, J=2.8, 1H, H-C(6) or H-

C(7)), 7.18-7.44 (m, 9H, arom. H), 8.98 (s, 1H, amidine-H); ^{13}C-NMR ($CDCl_3$): 34.45 (q, N-Me), 41.46 (t, C(2')), 41.81 (q, N-Me), 55.24 (q, O-Me), 63.67 (t, C(5')), 71.29 (d, C(3')), 83.84 (d, C(1') or C(4')), 86.01 (d, C(1') or C(4')), 86.54 (s, Cq-Trityl), 107.09 (d, C(6)), 113.17 (d, C(2")), 114.86 (d, C(7)), 126.86 (s, C(4'")), 127.85 and 128.33 (d, C(2'") and C(3'")), 130.20 and 130.21 (d, C(3"a) and C3"b)), 135.69 and 135.80 (d, C(4"a) and C4"b)), 144.61 (s, C(1'")), 150.20 (s, C(2)), 154.65 (s, C(8a)), 158.51 (s, C(1")), 159.78 (d, CH-amidine), 165.01 (s, C(4)).

The paragraph beginning at line 10, page 51, has been amended as follows:

8-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-4-[(dimethylamino)methylidene]-imidazo[1,2-a]-1,3,5-triazin-2(8H)-one 3'-(2-cyanoethyl)-N,N-diisopropyl-phosphoramidite. 8-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-4-[(dimethylamino)methyliden]-imidazo[1,2-a]-1,3,5-triazin-2(8H)-one (125 mg, 0.20 mmol) was dried over P_2O_5 under high vacuum for 3 days, and then dissolved in abs. CH_2Cl_2 (1.0 mL). Diisopropylethylamine (137 μL, 0.80 mmol) was added, followed by dropwise addition at ~~r.t~~RT. of 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite (54 μL, 0.24 mmol). After 1.5 h, Et_3N (0.2 mL) was added, the resulting precipitate dissolved in the chromatography elution mixture (1.0 mL) and chromatographed through silica gel (10 g)(eluant: CH_2Cl_2 /MeOH 95:5, containing 1% Et_3N) to yield the product 8-[2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-4-[(dimethylamino)methylidene]-imidazo[1,2-a]-1,3,5-triazin-2(8H)-one 3'-(2-cyanoethyl)-N,N-diisopropyl-phosphoramidite (mixture of two diastereomers) as an amorphous white solid (109 mg, 66%). FAB-MS (3-NOBA): 847 (M^++Na), 825 (M^++1); ^{1}H-NMR: The numbering of the two diastereomeric phosphoramidites is arbitrary. ($CDCl_3$): 1.16-1.18 (m, 2x12H, Me-isopropyl), 2.42-2.65 (m, 2x4H, H-C(2') and H-(CH_2-CN)), 3.166 (s, 3H, N-Me), 3.167 (s, 3H, N-Me), 3.21 (s, 2x3H, N-Me), 3.30-3.34 (m, 2x1H, Ha-C(5')), 3.42 (dd, J=3.2, 10.5, 1H, Hb-C(5'a)), 3.48 (dd, J=3.0, 10.5, 1H, Hb-C(5'b)), 3.53-3.69 and 3.71-3.88 (m, 2x4H, H-(CH-isopropyl) and H-(CH_2-O-P)), 3.787, 3.792 and 3.793 (s, 2x6H, O-Me), 4.14-4.19 (m, 2x1H, H-C(4')), 4.65-4.71 (m, 2x1H, H-C(3')), 6.45-6.49 (m, 2x1H, H-C(1')), 6.81-6.85 (m, 2x4H, ortho-arom. H), 6.98 (d, J=2.8, 1H, H-C(6a) or H-C(7a)), 7.05 (d, J=2.8, 1H, H-C(6a) or H-C(7a)), 7.08-7.09 (m, 2H, H-C(6b) and H-C(7b)), 7.20-7.34 and 7.41-7.44 (m, 2x9H, arom. H), 8.99 and 9.00 (s, 2x1H, amidine-H); ^{13}C-NMR ($CDCl_3$): 20.20 (td, J=7.1, CH_2a-CN), 20.43 (td, J=7.2, CH_2b-CN), 24.47, 24.53, 24.55, 24.57, 24.59, 24.61, 24.63 and 24.64 (q, Me-isopropyl), 35.36 (q, N-Me), 40.05 (t, C(2')), 41.74 (q, N-Me), 43.16, 43.24, 43.26 and 43.34 (d, CH-isopropyl), 55.24 and 55.27 (q, O-Me), 58.18 (td, J=18.7, CH_2a-OP), 58.25 (td, J=18.5, CH_2b-OP), 63.95 and 63.23 (t, C(5')), 72.80 (dd, J=16.0, C(3a')), 73.60 (dd, J=17.1, C(3b')), 83.34 and 83.40 (d, C(1')), 85.30 (dd, J=6.1, C(4a')), 85.40 (dd, J=3.6, C(4b')), 86.61 and 86.62 (s, Cq-Trityl), 107.43 and 107.45 (d, C(6)), 113.20 (d, C(2")), 113.98 and 133.99 (d, C(7)), 117.49 and 117.75 (s, CN), 126.99 and 127.03 (s, C(4'")), 127.90, 128.24 and 128.31 (d, C(2'") and C(3'")), 130.15, 130.18 and 130.21 (d, C(3")), 135.49, 135.52, 135.54 and 135.57 (d, C(4")), 144.41 and 144.42 (s, C(1'")), 150.59 and

150.67 (s, C(2)), 154.40 and 154.41 (s, C(8a)), 158.62 and 158.63 (s, C(1")), 159.73 (d, amidine), 164.26 and 164.31 (s, C(4)); ^{31}P-NMR ($CDCl_3$): 148.8 and 149.0.

The paragraph beginning at line 25, page 56, has been amended as follows:

Deprotection and Purification of Oligoribonucleotides Containing Standard Nucleotides Only. Two RNA octamers were purchased from MWG-Biotech in their 5'-O-DMT- and 2'-O-Fpmp-protected form. Following the instructions of the manufacturer, the oligonucleotides were deprotected by incubation in the provided deprotection soln. (500 μL, pH 2-3) for 24 h at ~~r.t~~RT. The soln. was cooled to 0 °C., neutralized with Et_3N (60 μL) and microfiltrated prior to HPLC purification, which was performed as described above for the pyADD containing octaribonucleotides.